

9/1967

THE AUSTRALIAN NATIONAL UNIVERSITY
John Curtin School of Medical Research

Annual Report 1966
Department of Physiology

1.

STAFF

Professor:

Sir John Eccles, M.B., B.S. (Melb.), M.A., D.Phil.(Oxon.),
Hon.Sc.D.(Cantab.), Hon.D.Sc.(Tas. and Brit.Columbia),
Hon.LL.D.(Melb.), F.R.A.C.P., F.R.S.N.Z., F.A.A., F.R.S.
(until 31 August 1966).

Professor of Pharmacology and Acting Head of Department:

David Roderick Curtis, M.B., B.S. (Melb.), Ph.D., F.A.A.

Electronics Engineer:

John Saxon Coombs, M.Sc.(N.Z.).

Senior Fellow:

John Ingram Hubbard, B.Med.Sci.(N.Z.), M.A., B.M.,
B.Ch.(Oxon.), Ph.D.

Fellows:

Rosamond Margaret Mason, M.Sc.(N.Z.), Ph.D. (until 1 July
1966).

Russell Ian Close, M.Sc.(N.Z.), Ph.D.(Ill.).

Senior Research Fellow:

Jörgen Fex, B.A.(Stockholm), M.D.

(Nobel Institute) Docent (until 30 March 1966).

Research Fellows:

Kazuo Sasaki, M.D., Ph.D.(Kyoto) (until 13 August 1966).

Timothy John Biscoe, B.Sc., M.B., B.S.(Lond.) (until
9 June 1966).

Graham Allen Ross Johnston, M.Sc.(Syd.), Ph.D.(Cantab.).

Ronald Walter Ryall, B.Sc.(Lond.), Ph.D.

Visiting Fellows:

Piergiorgio Strata, M.D.(Pisa) (until 21 July 1966).

Donald Montague Lewis, B.A., M.B., B.Chir.(Cantab.),
M.R.C.S., L.R.C.P. (until 4 August 1966).

Rosamond Margaret Mason, M.Sc.(N.Z.), Ph.D. (from 1 August
1966).

Leonhard Hösli, M.D.(Basel) (from 28 November 1966).

Honorary Fellow:

William Chesney de Groat, B.Sc., M.Sc.(Philadelphia
College of Pharmacy), Ph.D.(University of Pennsylvania)
(from 23 April 1966).

Exchange Scholar:

(Moscow State University)

Lev Nikolaevich Vorobiev, Ph.D.(M.S.U.).

Research Assistants:

Nancy Enid Stone, M.Sc.(W.A.), Ph.D.(Cantab.)

Helen Jacqueline Lloyd, M.Sc.(Melb.)

Keith Charles Hendy, B.E.(Brisbane) (from 1 February 1966)

Marcus Devanandan, M.B., B.S.(Vellore), Ph.D. (from 1 May
1966).

Head Technician:

L.M. Davies, Dip. Mech. & Elect. Eng., A.M.I.I.T.A.,
M.Q.E.I.

RESEARCH WORK

Introduction

The work of the Department has continued to be concerned with an analysis of nerve pathways in the central nervous system, the function of identified types of nerve cells, a study of the nature of chemical substances used to transmit information between nerve cells, the factors associated with the release of these transmitter substances from presynaptic nerve terminals, the biophysics of muscle contraction and the design of electronic apparatus for use in these specialised projects. Brief outlines of current research projects are given below.

3.

Cerebral cortex (Biscoe, Curtis)

A study was commenced of pathways converging upon the cerebral cortex, some of which may be cholinergic, and of the inter-connections between cortical neurones. It has been shown that the large pyramidal cells, which give rise to the cortico-spinal pathway, can be inhibited as a consequence of the chemical excitation of nearby interneurones. Thus some interneurones within the cortex are of the inhibitory type, and a detailed histological analysis of the distribution of these cells is in progress. A pharmacological analysis of the excitation of pyramidal tract neurones by impulses in pathways arising from specific thalamic nuclei has indicated that acetylcholine is unlikely to be involved as an excitatory transmitter substance released upon the cortical neurones in this system.

The cerebellum (Eccles, Sasaki, Strata)

During the year experiments were especially directed towards the detailed mechanism of the glomeruli in the granular layer of the cerebellum. These are of great importance as relay stations for the main input of information to the cerebellar cortex via the mossy fibres. It was shown that this mossy fibre input excited both granule cells and those Golgi cells which contribute dendrites to the glomeruli. It was further shown that the Golgi cell axons very effectively inhibited the granule cell discharges evoked by the mossy fibre excitation.

There was a systematic investigation of the potential profiles evoked by various mossy fibre inputs at all levels of the cerebellar cortex. It is possible to explain all details of these potentials in terms of the various synaptic excitatory and inhibitory actions by the various components of the cerebellar cortex.

Another investigation was designed to discover the field of action produced by a sharply focussed mossy fibre input. Inhibition was shown to extend for about 1 mm on either side of the excited beam of parallel fibres, a finding which is in accord with the histologically determined distribution of the inhibitory basket and stellate cells.

The inferior olivary nucleus (Armstrong, Harvey)

The inferior olivary nucleus of the brain stem has been studied in its role as a precerebellar relay nucleus. This nucleus receives afferents from many parts of the central nervous system, and its output is distributed by the olivo-cerebellar fibres to the whole extent of the cerebellar cortex. The localisation in the olivo-cerebellar projection has been confirmed by a combination of electrophysiological and histological techniques. The afferent pathways from the spinal cord and cerebral cortex consist of slowly conducting nerve fibres, and impulses in both these pathways produce an initial excitatory response which is followed by prolonged inhibition of the olivary neurones. The relation of the activity of Purkinje cells to potentials recorded from the cerebellar surface has been studied, and it seems likely that the large surface potentials seen by previous workers after stimulation of the cerebral cortex or of hindlimb nerves were generated by activity mediated by the inferior olive.

Long latency spinal reflexes (Devanandan, Lewis,
Mason, Stenhouse)

Research has continued into the long latency reflexes evoked by stimulation of cutaneous or muscle afferent fibres and recorded from the nerves to hind limb flexor muscles, and occasionally from nerves to extensors, in cats anaesthetised with

chloralose. This reflex, which is abolished by section of the spinal cord, is diminished by barbiturates and urethane, and does not seem to be present in animals made decerebrate under ether. The reflex is associated with the presence of long latency excitatory potentials recorded from flexor motoneurones. An as yet incomplete investigation suggests that this reflex is mediated in part via fibres in the ipsilateral dorso-lateral column originating from the cortex and brain stem structures in the vicinity of the superior colliculus.

Pharmacology of spinal interneurones (Biscoe,
Curtis, Ryall)

Some spinal interneurones within lumbar segments can be depressed by microelectrophoretically administered nor-adrenaline, and a study has been made of the receptors associated with this effect. A variety of nor-adrenaline-like substances, and blocking agents of peripheral nor-adrenaline and adrenaline receptors, have been tested, and the central receptors have been shown to differ from those occurring in the periphery. It has not been possible to find a specific antagonist for the depressant action of nor-adrenaline which would be of assistance in comparing this depression with synaptic inhibition. Preliminary studies indicate that nor-adrenaline has an effect upon the membrane of some spinal motoneurones similar to that of inhibitory transmitters, namely an increase in the level of resting potential, and further investigation of these problems is required.

Spinal autonomic neurones (de Groat, Ryall)

A study is being made of the properties of spinal autonomic neurones. Sympathetic neurones of thoracic segments are excited by microelectrophoretically administered 5-hydroxy-tryptamine and depressed by nor-adrenaline, and this finding is

of considerable interest because of the possible function of nerve fibres which contain these substances. These fibres originate from neurones in the lower brain stem, and terminate in the vicinity of sympathetic neurones. A physiological study is also being carried out on nerve cells in the sacral segments of the spinal cord which innervate pelvic viscera, particularly the bladder.

Transmitter identification (Curtis, Häslü, .

G. Johnston, I. Johnston, Lloyd, Stone)

Physical and chemical methods for the extraction and ultimate isolation of transmitters stored within synaptic endings have been improved. Subcellular fractions prepared by homogenisation of brain under carefully controlled conditions are separated by density gradient centrifugation and characterised structurally by electron microscopy. Further purification by thin layer chromatography, thin layer electrophoresis, liquid ion-exchange extraction and electrodialysis through ion exchange membranes is followed by physico-chemical and pharmacological assays, particularly upon single neurones in the central nervous system. These latter assays are carried out by the electrophoretic ejection of ionized substances from the individual barrels of multibarrel glass micropipettes fashioned by the highly skilled glassblowers of the Workshop of the School. Techniques whereby intracellular records can be obtained whilst ejecting any of five different substances upon a single nerve cell have been developed.

Interest continues to be maintained in relatively simple amino acids. It has been confirmed that glycine may have an effect upon spinal motoneurones very similar to that of inhibitory transmitters, and an analysis of closely related amino acids is continuing. Structure-activity studies are being carried out upon analogues of gamma-amino-butyric acid and glutamic acid with

particular reference to the search for specific antagonists of the effects of these amino acids upon neurones. Structural analogues of β -N-oxalyl-L- α , β -diaminopropionic acid, a powerful amino acid excitant which appears to be associated with neurolathyrism, are being synthesised and examined for pharmacological activity. Brain extracts are also being examined for oxalyl derivatives and related enzymes.

Transmitter release (Hubbard, Jones,
Kwanbunbunphen, Landau)

Studies during the year have been related to the release of acetylcholine at the mammalian neuromuscular junction, particularly to the role that calcium ions play in both the release process and the coupling between nerve impulses and release of transmitter. It has been found that calcium acts by forming complexes, presumably with nerve terminal constituents. Other ions inhibit (magnesium and sodium) or activate (potassium) the calcium action. Magnesium also forms complexes which can separately influence release. The time course of calcium action indicates that movement of calcium across the nerve cell membrane, perhaps in a complexed form, is associated with release. Experiments on the thermo-dynamic and osmotic aspects of the release process were continued during the year and investigation has begun into the coupling process which is believed to involve depolarization of the nerve terminals. It has been found that the quantitative relationship between release and depolarization is not, as was previously thought, sufficient to explain the coupling process.

Electronmicroscopy of motor nerve terminals has continued during the year and has been devoted particularly to the distribution of synaptic vesicles, 500 Å clear spherical structures found adjacent to the presumed chemical releasing membrane and thought to contain transmitter. Preliminary

observations indicate that there are special areas where vesicles come into intimate relationship with the membrane. It is hoped to correlate the numbers of vesicles in these special areas with electrophysiological studies of presynaptic release mechanisms.

Muscle contraction (Close, Hoh)

Neural control of the speed of muscle contraction has been investigated in various mammalian and amphibian fast and slow muscles. Measurements have been made of the force-velocity properties of the contractile material and the degree of activation of muscle fibres in both normal muscle and in muscle after cross-union of motor nerves and operative alterations of the central nervous system.

Electronics (Coombs, Hendy)

A study has been made of the measurement of noise in high impedance recording systems; satisfactory procedures for measuring the noise in active devices have been achieved, and the results obtained indicate the possibility of improved input stages for recording biological signals.

A separate project with considerable implications started as a small investigation to design a symmetrical driver circuit for a diodegate (a high-speed switch). The system can also be used as the basic circuit of a level-crossing detector with small hysteresis. As an application, the equipment has been used in an integrator unit suitable for neurophysiological research applications; rearrangement of the individual sections of the integrator produces a stimulator control system suitable for neurophysiological research. Further circuits have been designed for the possible development of a new stimulating and recording system to replace that originally designed and built

in 1951-52, and used continuously in each of the laboratories of the Department since that time. The new design, using solid-state devices, retains the good operational features of the old equipment, many of which are not obtainable in commercially available apparatus, but also adds extra facilities which experience has shown to be desirable.

Throughout the year the majority of the electronic technical staff have been engaged upon the design and construction of stimulating and recording equipment for an additional neuropharmacological laboratory. This project has been supported by the National Institute of Mental Health and Blindness, Washington.

TEACHING AND OTHER ACTIVITIES

Eight students are proceeding to the degree of Doctor of Philosophy, Dr. S.F. Jones, Miss S. Kwanbunbumpen, Dr. I.H. Johnston and Dr. J.F.Y. Hoh having commenced their courses during the year. The following students successfully completed the requirements for, and were awarded, the degree of Doctor of Philosophy: Dr. J.M. Crawford, Dr. M. Devanandan, Dr. P.W. Gage, Dr. Y. Løyning and Dr. R. Llinas.

Weekly departmental seminars have been held throughout the year together with frequent less formal discussions of research in progress. In August members of the department contributed papers to the meeting of the Australian Physiological Society held at the University of Queensland. Professor D.R. Curtis, Dr. G.A.R. Johnston and Dr. R.W. Ryall delivered a series of lectures on neurochemistry and neuropharmacology to Chemistry IV students in the School of General Studies.

Professor Sir John Eccles resigned from the Chair of Physiology on 31 August 1966 to take up an appointment as a

Member of the Institute for Biomedical Research in Chicago, U.S.A. Prior to his resignation Sir John was overseas on several occasions and attended the work session on Cerebellar Circuitry, Neurosciences Research Program, Boston; a conference on Neurophysiology in relation to Anaesthesiology, Seattle; the 16th Nobelpreistragertagung, Lindau; and the work session of the Neurosciences Research Program, Boulder. Sir John also gave three lectures as Visiting Professor, Davis Campus, University of California. Sir John Eccles had the honorary degree of Doctor of Science conferred upon him by the University of British Columbia, was elected a Foreign Associate of the United States National Academy of Sciences and was made an Honorary Professor of Northwestern University.

Professor D.R. Curtis was invited to present a paper at the Fourth International Meeting of Neurobiologists held in Stockholm. He also attended the Twelfth International Meeting of Neuropsychologists held in Athens, and lectured at the National University of Athens, the University of Oslo, the Jan Swammerdam Institute, Amsterdam, and was able to visit laboratories in Stockholm, London, Cambridge and Vancouver. In June Professor Curtis was advised of an award of US \$11,111 from the National Institute of Neurological Disease and Blindness, Washington, to assist in staffing and equipping an electropharmacological research laboratory, and in October a grant of A \$10,370 was received from the Wellcome Trust in order to purchase storage and computing equipment for the processing of neurophysiological data.

Mr. J.S. Coombs gave two lectures in the Department of Electrical Engineering during a visit to Monash University in August.

Dr. J. Hubbard spent 10 weeks with the Physiological Expedition to the Great Barrier Reef organized by the Scripps

Institute of Oceanography. He made a study of neuromuscular mechanisms of echinoderms and also of the properties of muscles of the ghost crab, Ocypode ceratophalma. Dr. Hubbard was an invited speaker at a conference organized by the New York Academy of Sciences on "Molecular aspects of cholinergic mechanisms". He also visited and lectured in biology departments of Northwestern, Purdue, Indiana, Southwestern, Stanford and New York State Universities.

Dr. R. Close was invited to a meeting held in New York which was organized by the Muscular Dystrophy Associations of America, and entitled "Exploratory concepts in muscular dystrophy". He was able to visit laboratories at New York, Syracuse and Urbana.

Dr. Rosamond Mason returned in February after spending three months in Japan and three months in England where she worked with Professor Charles Phillips, F.R.S. and Dr. Wu in the Department of Physiology, Oxford University.

PUBLICATIONS

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Expl. Brain Res.

- 1: Visiting research worker
- 2: A member of the Department of Experimental Pathology
- 3: A member of the Department of Physiology, Oxford